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(54) Title: OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF PATHOGEN-INDUCED EXPRESSION OF A
HETEROLOGOUS HYPERSENSITIVE RESPONSE ELICITOR

(57) Abstract: The present invention relates to a chimeric gene that includes a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA molecule. Also disclosed are an expression system and a host cell containing the chimeric gene. The present invention also relates to a transgenic plant resistant to disease resulting from oomycete infection, the transgenic plant including the chimeric gene. The present invention also relates to the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete. Transgenic seeds and transgenic cultivars obtained from the transgenic plant are also disclosed. Additional aspects of the present invention include methods of making a recombinant plant cell and a transgenic plant.



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OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF PATHOGEN-INDUCED EXPRESSION OF A HETEROLOGOUS HYPERSENSITIVE RESPONSE ELICITOR

5 This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/178,565, filed January 26, 2000, which is hereby incorporated by reference in its entirety.

This invention was made in part with support by the U.S. Government under Grant No. 97-34367-3937 from the U.S. Department of Agriculture. The U.S.
10 Government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to transgenic plants resistant to oomycete
15 infection which contain a heterologous hypersensitive response elicitor under the control of a promoter responsive to infection by an oomycete.

BACKGROUND OF THE INVENTION

20 In general, fungal plant diseases can be classified into two types: those caused by soilborne fungi and those caused by airborne fungi. Soilborne fungi cause some of the most widespread and serious plant diseases, such as root and stem rot caused by *Fusarium spp.* and root rot caused by *Phytophthora spp.* For example, *Phytophthora parasitica* var. *nicotiana*, a soilborne oomycete found in many tobacco growing regions worldwide, causes black shank, a highly destructive root and stem rot disease of many varieties of cultivated tobacco.

25 Since airborne fungi can be spread long distances by wind, they can cause devastating losses, particularly in crops which are grown over large regions. A number of pathogens have caused widespread epidemics in a variety of crops.

30 Important diseases caused by airborne fungi are stem rust (*Puccinia graminis*) on wheat, corn smut (*Ustilago maydis*) on corn, and late blight disease (*Phytophthora infestans*) on potato and tomato. *Plasmopera viticola* is an airborne oomycete that causes downy mildew disease on grape vines. The blue mold fungus (*Peronospora*

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tabacina) has caused catastrophic losses in tobacco crops, particularly in the United States and Cuba.

Most of these fungal diseases are difficult to combat, and farmers and growers must use a combination of practices, such as sanitary measures, resistant 5 cultivars, and effective fungicide against such diseases. Hundreds of millions of dollars are spent annually for chemical control of plant-pathogenic fungi. As a result, there is today a real need for new, more effective and safe means to control plant-pathogenic fungi, particularly oomycetes which are responsible for major crop loss.

Genetic engineering promises to be an effective strategy for reducing 10 the losses associated with diseases of field crops. Several successful approaches have been reported where the constitutive expression of antimicrobial peptides such as cecropins (Arce et al., "Enhanced Resistance to Bacterial Infection by *Erwinia Carotovora* Susp. *Atroseptica* in Transgenic Potato Plants Expressing the Attacin or the Cecropin SB-37 Genes," Am. J. Potato Res. 76:169-177 (1999)), lysozyme 15 (Nakajima et al., "Fungal and Bacterial Disease Resistance in Transgenic Plants Expressing Human Lysozyme," Plant Cell Reports 16:674-679 (1997)), and monoclonal antibodies (Tavladoraki et al., "Transgenic Plants Expressing a Functional Single Chain FV Antibody are Specifically Protected from Virus Attack," Nature 366:468-472 (1993)) effectively protected plants from parasitic organisms. However 20 successful, these approaches have limited application to food production since many of these antimicrobial peptides and plant defense molecules are potentially toxic or allergenic to humans (Franck-Oberaspach et al., "Consequences of Classical and Biotechnological Resistance Breeding for Food Toxicology and Allergenicity," Plant Breeding 116:1-17 (1997)). Thus, alternative approaches for genetically engineering 25 disease resistance would be more desirable.

Plants possess a highly evolved pathogen surveillance system which allows for recognition of specific pathogen derived molecules known as elicitors. Elicitor recognition results in an incompatible plant-microbe interaction, defined as the rapid activation of plant defense genes, typically resulting in the hypersensitive 30 response and the onset of systemic acquired resistance.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z.,

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"Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al., "Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, (1982), these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants

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and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas Syringae pv. Syringae Harpin_{PSS}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); Wei, Z.-M., et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M., et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of Pseudomonas solanacearum," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 *PopA1* protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al., "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of Pseudomonas solanacearum," EMBO J. 13:543-53 (1994)). However, *P. solanacearum* *popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin_{Ech}: Soft-Rot Pathogenesis," MPMI 8(4): 484-91

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(1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1966)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

Because the hypersensitive response results in localized necrosis of plant tissue, it is desirable to limit expression of a heterologous hypersensitive response elicitor to certain tissues in transgenic plants. This approach is discussed generally in PCT publication WO 94/01546 to Beer et al., but no specific transgenic plants are identified and only two suitable fungus-responsive promoters are suggested, e.g., the phenylalanine ammonia lyase and chalcone synthase promoters. No promoters responsive specifically to infection by oomycetes are identified therein.

The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

The present invention relates to a chimeric gene that includes a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA molecule. Also disclosed are an expression system that includes a vector in which is inserted a chimeric gene of the present invention and a host cell that includes a chimeric gene of the present invention.

Another aspect of the present invention relates to a transgenic plant resistant to disease resulting from oomycete infection. The transgenic plant includes a chimeric gene of the present invention, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete.

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Transgenic seeds and transgenic cultivars obtained from the transgenic plant are also disclosed.

An additional aspect of the present invention relates to a method of making a recombinant plant cell. This is accomplished by transforming a plant cell 5 with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter.

A further aspect of the present invention relates to a method of making a plant resistant to disease resulting from oomycete infection. This is accomplished 10 by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and regenerating the plant from the transformed plant cell.

The present invention confers oomycete-induced disease resistance to 15 plants transformed with a chimeric gene encoding a hypersensitive response elicitor protein or polypeptide, which is transcribed within a limited population of plant cells in response to infection of the plant by an oomycete. To limit transcription of the chimeric gene within a certain population of plant cells, the chimeric gene includes a promoter that is responsive to infection by an oomycete (i.e., it is activated by the 20 oomycete). The hypersensitive response elicitor protein or polypeptide can cause tissue collapse at the site of infection and/or induce systemic resistance against the oomycete and other pathogens. By using the promoter from the potato *gst1* gene, for example, which is activated by infection with oomyceteous fungi, the present 25 invention can control fungal pathogens within crops without harming the transgenic plant and without resorting to use of environmentally damaging chemicals.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation and partial restriction map of T- 30 DNA in plant transformation vector pCPP1294. Filled triangles represent the left and right borders; *Pgst1* represents the *gst1* promoter from potato variety Atlantic; PR1-b represents the DNA molecule encoding a signal sequence from *Nicotiana tabacum*;

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hrpN represents the DNA molecule encoding the hypersensitive response elicitor harpin_{Ea} of *Erwinia amylovora*; NT represents the nos terminating region; *aacC1* represents the gentamycin resistance cassette.

Figure 2 is an image of transgenic *Arabidopsis* plants containing a construct encoding GUS under control of the *gst1* promoter. To demonstrate pathogen inducibility of the *gst1* promoter in *Arabidopsis*, GUS staining was measured following inoculation of the plants with water (left) or *P. parasitica* (right). GUS expression is indicated by dark staining.

Figures 3A and 3B show an analysis of *hrpN* gene expression in *Arabidopsis* transgenic line GSSN8-4, containing the construct shown in Figure 1, after inoculation with *P. parasitica* NOCO. At one day intervals leaves were collected for isolation of total RNA. Figure 3A is a Northern blot analysis performed using *hrpN* DNA as a probe. Figure 3B is an ethidium bromide stained gel shown as a control (bottom).

Figures 4A and 4B are images demonstrating *Arabidopsis* GSSN 8-4 are resistant to *P. parasitica*. Figure 4A shows the effects of *P. parasitica* infection in WT *Arabidopsis* (control, left) and GSSN 8-4 *Arabidopsis* (test, right). Figure 4B shows the degree of trypan blue staining of *P. parasitica*-infected leaves of WT (control, left) and GSSN 8-4 plants (test, right), both taken 10 days post-inoculation.

Figure 5 is a graph depicting the severity of *P. parasitica* infection in WT (control), EV (control), and *hrpN* transgenic plants (test). Two week old plants were drop inoculated with conidiospores of *P. parasitica* (2 ml drops; 5×10^4 spores/ml). Ten days after inoculation, 30 plants of each genotype were rated for disease severity. Ratings were adapted from Cao et al. ("Generation of Broad-Spectrum Disease Resistance by Overexpression of an Essential Regulatory Gene in Systemic Acquired Resistance," *Proc. Natl. Acad. Sci. USA* 95:6531-6536 (1998), which is hereby incorporated by reference) as follows: 1, no conidiophores present on plant; 2, 0-5 conidiophores per infected plant; 3, 6-20 conidiophores present on a few infected leaves; 4, 6-20 conidiophores present on most infected leaves; 5, more than 20 conidiophores on all infected leaves.

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the present invention relates to a novel DNA construct in the form of a chimeric gene. The chimeric gene includes a first DNA molecule 5 encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA molecule. As discussed more fully hereinafter, a chimeric gene of the present invention is particularly useful in preparing 10 a transgenic plant for the purpose of rendering the transgenic plant resistant to disease resulting from infection thereof by an oomycete.

The first DNA molecule can encode any hypersensitive response elicitor protein or polypeptide which is effective in triggering a hypersensitive response (i.e., in a particular host plant selected for transformation). Generally, it is 15 desirable to express hypersensitive response elicitors only in plants which are non-hosts for the source organism of the hypersensitive response elicitor. Suitable hypersensitive elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides 20 from bacterial sources include, without limitation, the hypersensitive response elicitors from *Erwinia* species (e.g., *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*, *Pseudomonas solanacearum*, etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from 25 these Gram-negative bacteria, it is possible to use elicitors from Gram-positive bacteria. One example is the hypersensitive response elicitor from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors 30 (i.e., elicins) from various *Phytophthora* species (e.g., *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

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Preferably, the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide of *Erwinia chrysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*, or *Pseudomonas solanacearum*.

- The hypersensitive response elicitor protein or polypeptide from
 5 *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1
 as follows:

	Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser	15
1	5	10
10	Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser	30
	20	25
	<u>Leu</u> <u>Gly</u> Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr	45
	35	40
15	Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu	60
	50	55
	Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser	80
	65	70
	Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys	95
20	85	90
	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp	110
	100	105
	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln	125
	115	120
25	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met	140
	130	135
	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly	160
	145	150
	Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly	175
	165	170
30	Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu	190
	180	185
	Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala	205
	195	200
35	Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val	220
	210	215
	Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp	240
	225	230
		235

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15 This hypersensitive response elicitor protein or polypeptide has a molecular weight of
34 kDa, is heat stable, has a glycine content of greater than 16%, and contains
substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor
protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence
20 corresponding to SEQ. ID. No. 2 as follows:

	cgattttacc cgggtgaacg tgctatgacc gacagcatca cggttattcga caccgttacg	60
	gcgttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgcccga atccggcgtc	120
	gatctggat ttcaagtggg ggacaccggg cgtgaactca ttagtgcagat tcagccgggg	180
25	cagcaatac ccggcatgtt ggcacgcgtg ctgcgtcgct gttatcagca ggcggcagag	240
	tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg	300
	ccgtcgatc ccggcagttt tccgcagggtg atcgaacgtt tggttgaact ggcgggaatg	360
	acgttgccgt cgctatccat agcaccgacg ggcgtccgc agacaggaa cggacgcgcc	420
	cgatcattaa gataaaggcg gctttttta ttgcaaaaacg gtaacggtga ggaaccgttt	480
30	caccgtcgcc gtcactcagt aacaagtatc catcatgatg cctacatcg gatcggcggt	540
	ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca	600
	aattacgatc aaagcgcaca tcggcggtga tttgggcgtc tccggctctgg ggctgggtgc	660
	tcagggactg aaaggactga attccgcggc ttcatcgctg ggttccagcg tggataaaact	720
	gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg ggcgcgcgt	780
35	ggcgcagggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tggccagtc	840
	tttcggcaat ggccgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat cggccggcga	900

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tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960
 caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020
 ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080
 caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctgggg caggcggctt 1140
 5 gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200
 ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260
 ccgccactt gtagataaaag aagatgcgg catggcggaa gagatcggcc agtttatgga 1320
 tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380
 gacggacgac aaatcctggg cttaaagcgct gагтaaaccg gatgatgacg gтatgaccgg 1440
 10 cgccagcatg gacaaattcc gtcagggcat gggtatgatc aaaagcgccgg tggcgggtga 1500
 taccggcaat accaacctga acctgcgtgg cgccggcggt gcatcgctgg gтatcgatgc 1560
 ggctgtcgtc ggcgataaaa tagccaaacat gtcgctgggt aagctggcca aegcctgata 1620
 atctgtgctg gcctgataaaa gcgaaacga aaaaagagac gggaaagcct gтctctttc 1680
 ttattatgctg gtttatgctg ttacctggac cggttaatca tcgtcatcgta tctggtacaa 1740
 15 acgcacattt tcccggtcat tcgcgtcggt acgcgcacata tcgcgtatgg catcttcctc 1800
 gtcgctcaga ttgcgcggct gatggggAAC gcccgggtgg atatagagaa actcgccggc 1860
 cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gccccctttag 1920
 cagatagatt gcggtttcgt aatcaacatcg gtaatcggtt cccgcgtgg cgccggccgg 1980
 gatcaccacaa atattcatag aaagctgtct tgacacctacc gтatcgccgg agataaccgac 2040
 20 aaaatagggc agttttgcg tggtatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100
 gttcgcatc atctttctcc atctggcga cctgatcggt t 2141

The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.

25 No. 3 as follows:

	Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
	1 5 10 15
	Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
30	20 25 30
	Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Asn
	35 40 45
	Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
	50 55 60

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	Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu			
65	65	70	75	80
	Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu			
	85	90	95	
5	Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr			
	100	105	110	
	Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro			
	115	120	125	
10	Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser			
	130	135	140	
	Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln			
	145	150	155	160
	Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly			
	165	170	175	
15	Gln Asp Gly Thr Gln Gly Ser Ser Gly Gly Lys Gln Pro Thr Glu			
	180	185	190	
	Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly			
	195	200	205	
20	Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly			
	210	215	220	
	Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu			
	225	230	235	240
	Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln			
	245	250	255	
25	Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln			
	260	265	270	
	Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe			
	275	280	285	
30	Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met			
	290	295	300	
	Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro			
	305	310	315	320
	Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser			
	325	330	335	
35	Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn			
	340	345	350	
	Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn			
	355	360	365	

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Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
 370 375 380
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
 385 390 395 400

5 Gly Ala Ala

This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

	aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa	60
	gaggaatacg ttatgagtct gaataacaagt gggctggag cgtcaacgt gcaaattct	120
	atccggcgtg cgggcgaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg	180
20	ggtggcaatt ctgcactggg gctggcggc ggtaatcaaa atgataccgt caatcagctg	240
	gctggcttac tcaccggcat gatgatgatg atgagcatga tggcgggtgg tgggctgatg	300
	ggcggcgtggct taggcgggtgg cttaggtaat ggcttgggtg gtcagggtgg cctggcggaa	360
	ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa	420
	ggcggcaaca ataccacttc aacaacaat tccccgtgg accaggcgct gggtattaac	480
25	tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgcac	540
	cccgatgcgc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggtgatggg	600
	caagatggca cccagggcag ttccctctggg ggcaagcgc cgaccgaagg cgagcagaac	660
	gcctataaaa aaggagtcac tgatgcgtg tcgggcctga tggtaatgg tctgagccag	720
	ctccttggca acggggact gggaggtggt cagggcggta atgctggcac gggcttgcac	780
30	gttcgtcgc tggcggcaa agggctgcaa aacctgagcg ggccgggtgg ctaccagcag	840
	ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat	900
	atccggtaacgc acaggcacag ttcaaccgt tcttcgtca ataaaggcga tcgggcgtg	960
	gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac	1020
	cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catggcAAA agcactgagc	1080

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aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaaggc 1140
 atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200
 ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260
 cttggcaagc tggcgccggc ttaagctt 1288

5

The hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

10	Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met 1 5 10 15
	Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser 20 25 30
15	Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met 35 40 45
	Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala 50 55 60
20	Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Ile Glu Asp Val 65 70 75 80
	Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe 85 90 95
	Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met 100 105 110
25	Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu 115 120 125
	Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met 130 135 140
30	Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro 145 150 155 160
	Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe 165 170 175
	Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile 180 185 190
35	Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly 195 200 205
	Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser 210 215 220

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	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser
																240
	225															
																235
																255
																245
																250
5																
																270
																260
																275
																285
																280
10																
																300
																295
																320
																315
																310
																335
																325
15																
																340

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

20 Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "Pseudomonas syringae pv. *syringae* Harpin_{Ps}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. 25 ID. No. 6 as follows:

	atgcagagtc	tcagtcttaa	cagcagctcg	ctgcaaacc	cggcaatggc	ccttgcctg	60
	gtacgtcctg	aagccgagac	gactggcagt	acgtcgagca	aggcgcttca	ggaagttgtc	120
30	gtgaagctgg	ccgaggaact	gatgcgaat	ggtcaactcg	acgacagctc	gccattggga	180
	aaactgttgg	ccaagtcgat	ggccgcagat	ggcaaggcgg	gccccggat	tgaggatgtc	240
	atcgctgcgc	tggacaagct	gatccatgaa	aagctcggtg	acaacttcgg	cgcgtctgcg	300
	gacagcgcct	cgggtaccgg	acagcaggac	ctgatgactc	aggtgctcaa	tggcctggcc	360
	aagtgcgtgc	tctgatgatct	tctgaccaag	caggatggcg	ggacaagctt	ctccgaagac	420
35	gatatgccga	tgctgaacaa	gatcgcgac	ttcatggatg	acaatcccgc	acagtttccc	480
	aaggccgact	cgggcctcctg	ggtgaacgaa	ctcaaggaag	acaacttcct	tgatggcgac	540

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	gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag	600
	agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagttttcc	660
	aacaactcggtt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc	720
	ggcaatacccg tgggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa	780
5	tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtacgtcg	840
	gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tggcggctt gctgctcaag	900
	ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct	960
	gchgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca	1020
	gcctga	1026

10

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference.

15

The hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

	Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln	
	1 5 10 15	
20	Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser	
	20 25 30	
	Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile	
	35 40 45	
25	Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly	
	50 55 60	
	Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala	
	65 70 75 80	
	Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser	
	85 90 95	
30	Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met	
	100 105 110	
	Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala	
	115 120 125	
35	Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val	
	130 135 140	
	Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala	
	145 150 155 160	

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Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
 165 170 175
 Gly Ala Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190
 5 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220
 10 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln
 260 265 270
 15 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
 290 295 300
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320
 20 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335
 Gln Ser Thr Ser Thr Gln Pro Met
 340

25 Further information regarding this hypersensitive response elicitor protein or
 polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al.,
 "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia
 Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO
 30 J. 13:543-533 (1994), which is hereby incorporated by reference. It is encoded by a
 DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence
 corresponding SEQ. ID. No. 8 as follows:

atgtcagtcg gaaacatcca gagcccgctcg aacctccccgg gtctgcagaa cctgaacctc 60
 35 aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgtat caagcaggtc 120
 gagaaggaca tcctcaacat catcgagcc ctcgtgcaga aggccgcaca gtcggcgggc 180
 ggcaacaccg gtaacaccgg caacgcgcgg gcgaaggacg gcaatgccaa cgccggcgcc 240

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	aacgaccgcga gcaagaacga cccgagcaag agccaggctc cgcatcgcc caacaagacc	300
	ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa	360
	gacctggtga agctgctgaa ggccgcctg cacatgcagc agcccgccgg caatgacaag	420
	ggcaacggcg tggcggtgc caacggcgcc aagggtgcgg gcccgcaggg cggcctggcc	480
5	gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcccgcggcgg tgctggcgcc	540
	ggccgcggcgg gtggcggtgt cggcgggtgt ggtggcgccgg atggcggtc cggtgcgggt	600
	ggcgcaggcg gtgcgaacgg cggcgcggc ggcaatggcg tgaacggcaa ccaggcgaac	660
	ggcccgccaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac	720
	caggcgccgc tcacccggcgt gctgaaaaag ctgtatgaaaga tcctgaacgc gctggtgca	780
10	atgatgcagc aaggcgccct cggcggcgcc aaccaggcgc agggcggtc gaagggtgccc	840
	ggcaacgcct cggcggcttc cggcgcgaac cccggcgcga accagccccgg ttcggcgat	900
	gatcaatgtt cccggccagaa caatctgaa tcccaagatca tggatgtggtaa gaaggaggtc	960
	gtccagatcc tgcagcagat gctggcgccg cagaacggcg gcagccagca gtccacctcg	1020
	acgcagccga tgtaa	1035

15

Other embodiments of the present invention include, but are not limited to, use of the nucleotide sequence encoding for the hypersensitive response elicitor protein or polypeptide from *Erwinia carotovora* and *Erwinia stewartii*.

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp N*_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

The hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et al., "Structure and Activity of Proteins from Pathogenic Fungi

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- Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants." Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.
- Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

Other elicitors can be readily identified by isolating putative hypersensitive response elicitors and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art. The isolated DNA molecule can then be introduced into the chimeric gene for expression in a transgenic plant of the present invention.

The first DNA molecule can also encode fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding

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editions), which are hereby incorporated by reference. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., *Science* 257: 85-88 (1992), which is hereby incorporated by reference.

- 5 In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," *Science* 252:1643-51 (1991), which is hereby incorporated by reference.
- 10 These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The 15 fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. 20 ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. 25 DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

The first DNA molecule also can be a DNA molecule that hybridizes under stringent conditions to the DNA molecule having nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, or 8. An example of suitable stringency conditions is when 30 hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the

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temperature for either hybridization or washing conditions or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous 5 RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% 10 ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 µg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

Variants of suitable hypersensitive response elicitor proteins or polypeptides can also be expressed by the first DNA molecule. Variants may be 15 made by, for example, the deletion, addition, or alteration of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated 20 to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide (such as a 6xHis tag).

The promoter of the chimeric gene should be selected on the basis of its ability to induce transcription of the first DNA molecule in response to infection of the plant by an oomycete (i.e., the oomycete activates the promoter).

25 According to one embodiment, the promoter preferably includes some or all of the promoter-effective regions of a *gst1* gene from potato. The *gst1* promoter is activated in response to infection by oomycetes and not by wounding or other environmental perturbations. The *gst1* promoter from potato has a nucleic acid sequence corresponding to SEQ. ID. No. 9 as follows:

30 gaattcagga agaattttgt aggttcaact aaatttatata tataatataaaaaaaa 60
aattattaga cgcttcgact atttacttac tttaaaattt gaattttcgta acgaataaaa 120
ttatggtca gagaaaagtc ttttagctat tcacatgcta ggaagttca cttttggcgg 180

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	atcagtgatt gtatattatt taatataatat caatttctc atcaaactga aaatgaaaga	240
	taaaattaat attaaaaact ccattcattt taatttattt tcatgtttt acttgatcca	300
	aatctaca atttaaaagg ttttaaattt ttgtgcttt ttttaaatta aaaatatgtc	360
	aatatatta aaatatattt tttaaatttt atactaaaaa acatgtcaca tgaatatttg	420
5	aaattataaa attatcaaaa ataaaaaaag aatatttctt taacaaatta aaattgaaaa	480
	tatgataaat aaattaaaact attctatcat tgattttct agccaccaga tttgacccaaa	540
	cagtgggtga catgagcaca taagtcatct ttattgtatt ttattactca ctccaaaaat	600
	atagggaaata tgtttactac ttaatttagt caaatataat tttatattag aataattgaa	660
	tagtcaaaca agaaacttta atgcattcattt attttt	696
10	Effective fragments of SEQ. ID. No. 9 are also encompassed by the present invention. U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al.. which are hereby incorporated by reference, define promoter-effective regions of the potato <i>gst1</i> promoter. Preferably, the <i>gst1</i> promoter includes a nucleotide sequence corresponding, at a minimum, to nucleotides 295-567 of SEQ. ID. No. 9. The <i>gst1</i> promoter can also include effective portions containing nucleotides 295-696 of SEQ. ID. No. 9.	
15		
20		
25		
30		

The chimeric gene of the present invention also includes an operable 3' regulatory region, selected from among those which are capable of providing correct transcription termination and polyadenylation of mRNA for expression in plant cells, operably linked to the first DNA molecule which encodes for a hypersensitive response elicitor. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference). Virtually any 3' regulatory region known to be operable in plants would suffice for proper expression of the coding sequence of the chimeric gene of the present invention.

The first DNA molecule, promoter, and a 3' regulatory region can be ligated together using well known molecular cloning techniques described in

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Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference.

The chimeric gene can also include a second DNA molecule encoding a secretion signal. A number of suitable secretion signals are known in the art and other are continually being identified. The secretion signal can be an RNA leader which directs secretion of the subsequently transcribed protein or polypeptide, or the secretion signal can be an amino terminal peptide sequence that is recognized by a host plant secretory pathway. The second DNA molecule can be ligated between the promoter and the first DNA molecule, using known molecular cloning techniques as indicated above.

According to one embodiment, the second DNA molecule encodes a secretion signal derived from *Nicotiana tabacum*. Specifically, this DNA molecule encodes the secretion signal polypeptide for *PRI-b* gene of *Nicotiana tabacum*. This second DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

tctagaccat gggattttt ctctttcac aaatgccctc attttttctt gtgtcgacac 60
ttctcttatt cctaataata tctcactctt ctcatgcccc aaactctaga 110

The above sequence includes XbaI sites (underlined) at each end to facilitate insertion of the second DNA molecule into the chimeric gene of the present invention. The coding sequence of SEQ. ID. No. 10 starts at base 8. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

20	Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser 1 5 10 15
	Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn 20 25 30
	Ser Arg

An alternative second DNA molecule encoding the secretion signal polypeptide for *PRI-b* gene of *Nicotiana tabacum* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

- 24 -

atgggatttt ttctcttttc acaaatgccc tcatttttc ttgtctctac acttctctta 60
 ttcctaataa tatctcaactc ttctcatgcc caaaaactctc aa 102

- 5 This nucleotide sequence is disclosed in Genbank Accession No. X03465, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 13 as follows:

10 Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser
 1 5 10 15
 Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn
 20 25 30
 Ser Gln

15

Yet another second DNA molecule encodes the secretion signal for the *PR1-a* gene of *Nicotiana tabacum*. This DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 14 as follows:

20 atgggatttg ttctcttttc acaattgcct tcatttcttc ttgtctctac acttctctta 60
 ttcctagtaa tatccccactc ttgccgtgcc 90

- This DNA molecule is disclosed in Genbank Accession No. X06361, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu Leu Val Ser
 1 5 10 15
 30 Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg Ala
 20 25 30

- Still another second DNA molecule encodes the secretion signal for the *PR4-a* gene of *Nicotiana tabacum*. This DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 16 as follows:

40 atggagagag ttaataatta taagttgtgc gtggcattgt tgatcatcag catggtgatg 60
 gcaatggcgg cggca 75

- 25 -

This DNA molecule is disclosed in Genbank Accession No. X58546, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

10 Each second DNA molecule can be cloned using primers that introduce restriction sites at the 5' and 3' ends thereof to facilitate insertion of the second DNA molecule into the chimeric gene of the present invention. SEQ. ID. No. 10 is shown to include such restriction sites (e.g., XbaI).

Further aspects of the present invention include an expression system
that includes a vector containing a chimeric gene of the present invention, as well as a
host cell which includes a chimeric gene of the present invention. As described more
fully hereinafter, the recombinant host cell can be either a bacterial cell (i.e.,
Agrobacterium) or a plant cell. In the case of recombinant plant cells, it is preferable
that the chimeric gene is stably inserted into the genome of the recombinant plant cell.

The chimeric gene can be incorporated into cells using conventional recombinant DNA technology. Generally, this involves inserting the chimeric gene into an expression vector or system to which it is heterologous (i.e., not normally present). As described above, the chimeric gene contains the necessary elements for the transcription and translation in plant cells of the first DNA molecule (i.e., encoding the hypersensitive response elicitor protein or polypeptide) and, if present, the second DNA molecule.

U.S. Patent No. 4,237,224 issued to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Once the chimeric gene of the present invention has been prepared, it is ready to be incorporated into a host cell. Recombinant molecules can be introduced

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- into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Springs Laboratory, Cold Springs Harbor,
- 5 New York (1989), which is hereby incorporated by reference. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like. Preferably the host cells are either a bacterial cell or a plant cell.
- Accordingly, another aspect of the present invention relates to a method of making a recombinant plant cell. Basically, this method is carried out by
- 10 transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter. Preferably, the chimeric gene is stably inserted into the genome of the recombinant plant cell as a result of the transformation.
- 15 A related aspect of the present invention concerns a method of making a plant resistant to disease resulting from oomycete infection. Basically, this method is carried out by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and regenerating a plant
- 20 from the transformed plant cell.

One approach to transforming plant cells with a chimeric gene of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in

25 U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles

30 with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector

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and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the chimeric gene is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the chimeric gene. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The chimeric gene may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are 10 electroporated in the presence of plasmids containing the chimeric gene. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the chimeric gene into plant cells is to 15 infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the chimeric gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on 20 regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized 25 only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a chimeric gene of the present invention can be introduced into appropriate plant cells by means of the Ti plasmid of 30 *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into

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the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

5 After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the chimeric gene of the present invention. Suitable selection markers include, without limitation, markers 10 coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present 15 invention. Cells or tissues are grown on a selection media containing an antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present 20 invention relates to a transgenic plant that is resistant to disease resulting from oomycete infection. The transgenic plant includes a chimeric gene of the present invention, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete. Preferably, the chimeric gene is stably inserted into the genome of the transgenic plant of the present invention.

25 Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants. Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

30 It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce,

endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5 Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form
10 plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and
15 repeatable.

After the chimeric gene is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord
20 with common agricultural procedures known to those in the field.

Resistance against different types of oomycetes may be imparted to transgenic plants according to the present invention. Without being bound by any particular theory, it is believed that a hypersensitive response elicitor protein or polypeptide encoded by the first DNA molecule is transcribed in response to infection
25 of the plant by an oomycete. The exact mechanism by which the promoter is activated to regulate transcription of sequences under its control is not fully understood; however, the first DNA molecule is transcribed and the hypersensitive response elicitor is expressed in a limited population of cells (i.e., those in which transcription has been induced following oomycete infection). Once expressed, it is
30 believed that the hypersensitive response elicitor can either be secreted from the plant cell (assuming the chimeric gene also contains a second DNA molecule encoding an N-terminal secretion signal) or leaked from an oomycete-infected plant cell.

- 30 -

Regardless of how the hypersensitive response elicitor is delivered to the intercellular environment, it is believed that the hypersensitive response elicitor protein or polypeptide will initiate a hypersensitive response to cause localized necrosis of oomycete-infected tissues. In addition, systemic acquired resistance may be developed by the transgenic plant following initiation of the hypersensitive response. This may yield broad disease and/or pathogen resistance to the transgenic plants of the present invention.

Oomycetes against which resistance is imparted include, without limitation, species of *Plasmopara*, *Phytophthora*, *Peronospora*, *Pseudoperonospora*, *Bremia*, *Sclerospora*, *Aphanomyces*, *Pythium*, and *Albugo*.

According to one embodiment of the present invention, an oomycete resistant transgenic tobacco plant includes a chimeric gene of the present invention, wherein expression of the encoded hypersensitive response elicitor is responsive to infection of the plant by an oomycete that is a pathogen of tobacco, including, but not limited to, *Peronospora tabacina* (which causes blue mold) and *Phytophthora parasitica* (which causes black shank).

The chimeric gene of the present invention can be utilized to impart oomycete resistance for a wide variety of tobacco plants, some of which may possess varying levels of natural resistance against pathogenic oomycetes. The varieties of tobacco plants which can be protected include, without limitation, those referred to as Coker 371 Gold, K 149, K 326, K 346, K 394, K 730, RG 11, RG17, RG22, Speight G-70, Speight G-117, Speight G-126, GL939, NC 55, NC 71, NC 72, NC 95, NC 2326, OX 207, OX 940, RG 81, RG H4, RG H61, Speight 168, Speight NF3, Speight 172, CU 236, CU 387, CU 368, NC TG91, OX 4142NF, OX 4083, RG 4H2-12, RG 25 4H2-17, RG 4H2-20, Speight 177, Speight 178, Speight 179, VPI 107, VPI 605, NG TG94, KY 14, KY 8959, KY 907, KY 908, TN 86, TN 90, TN 97, VA 116, VA 509, B 21 x KY 10, KY 14 x L8, NC 3, NC BH129, DH332, COOP 313, COOP 543, Clay's 403, Clay's 502, HY 402, PF 561, and R 711.

According to another embodiment of the present invention, an oomycete resistant transgenic grape plant includes a chimeric gene of the present invention, wherein expression of the encoded hypersensitive response elicitor is responsive to infection of the plant by an oomycete that is a pathogen of grape,

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including, but not limited to, *Plasmopara viticola* (which causes downy mildew), *Pythium* spp. (which cause root and/or stem rot), and *Phytophthora* spp. (which cause root and/or stem rot).

The chimeric gene of the present invention can be utilized to impart 5 oomycete resistance for a wide variety of grapevine plants. The chimeric gene is particularly well suited to imparting resistance to *Vitis* scion or rootstock cultivars. Scion cultivars which can be protected include, without limitation, those commonly referred to as Table or Raisin Grapes, such as Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Cornish, Black Damascus, Black Malvoisie, 10 Black Prince, Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight, Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, 15 Malaga, Monukka, Muscat of Alexandria, Muscat Flame, Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, and Thomuscat. They also include, without limitation, those used in wine production, such as Aleatico, Alicante Bouschet, Aligote, 20 Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc, Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, 25 Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier, Mission, Montua de Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-George, Primitivo di Gioa, Red Veltliner, Refosco, Rkatsiteli, Royalty, 30 Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc, Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat,

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- Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Troussseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel. Rootstock cultivars which can be protected include Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, Vitis rupestris Constantia, *Vitis californica*, and *Vitis girdiana*.
- Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., scion or rootstock cultivars) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart oomycete resistance to plants.

EXAMPLES

- The following examples are provided to illustrate embodiments of the present invention, but they are by no means intended to limit its scope.

- Example 1 - Construction of Chimeric Gene**
- Cloning of *gst1* promoter
- The *gst1* promoter region from nucleotides (539 to +48) (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," *Mol. Gen. Genet.* 236 (2-3):179-86 (1993), which is hereby incorporated by reference), was PCR amplified using DNA from potato cultivar Atlantic, using a forward primer containing a BamHI site (SEQ. ID. No. 18) as follows:

- 33 -

tgacggatcc taggaagttt cactttgggt gg

32

a reverse primer containing an EcoRI site (SEQ. ID. No. 19) as follows:

5 tagcgaattc tatgtgtggt tggctccct tg

32

and PrimeZyme DNA polymerase (Whatman Biometra, Goettingen, Germany). The DNA was ligated into the LITMUS 38 vector (New England Biolabs, Beverly, MA) and three clones were sequenced on an ABI 377 sequencer at the Cornell 10 BioResource Center. Each clone had two to three nucleotide changes when compared to the published sequence (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," *Mol. Gen. Genet.* 236: (2-3) 179-86 (1993), which is hereby incorporated by reference). The changes were most likely due to mistakes made by 15 the polymerase because the promoter is extremely A-T rich and all but one of the changes were in different places in the three clones. One clone, pCPP1308, with a single change in the cis-acting region identified by Martini et al. ("Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," *Mol. Gen. Genet.* 236: (2-3) 179-86 (1993), 20 which is hereby incorporated by reference) was used as the source of the *gst1* promoter in all subsequent constructions.

Plant Transformation Constructs

The *gst1:uidA* construct was made by ligating the *gst1* promoter from 25 pCPP1308 into pBI101 (Clontech Labs, Palo Alto, CA). For the *gst1:hrpN* and *gst1:signal sequence:hrpN* constructs (described below), the *gst1* promoter region was engineered to have a 5' HindIII site and a 3' XbaI site by the polymerase chain reaction (PCR) using pCPP1308 as the template. The forward primer had the nucleotide sequence of SEQ. ID. No. 18 and the reverse primer had a nucleotide 30 sequence according to SEQ. ID. No. 20 as follows:

32

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For *gst1:hrpN* constructs, the *hrpN* gene of *Erwinia amylovora* (i.e., encoding a hypersensitive response elicitor identified as harpin_{Ea}) was engineered to have a 5' XbaI restriction site and a 3' SstI restriction site by PCR using pCPP1084 (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia Amylovora*," *Science* 257:85-88 (1992), which is hereby incorporated by reference) as the template. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 21 as follows:

10 atactctaga accatgggtc tgaataacaag tggg

34

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

15 tcatgagctc ttaagccggc ccagcttgcc aagtgc

35

For *gst1:signal sequence:hrpN*, the *hrpN* gene was engineered to have a BamHI site on each end. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 23 as follows:

20 tagaggatcc ctgaatacaa gtgggctggg agcg

34

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

25 tcatggatcc ttaagccgcg cccagcttgc caagtgc

36

The nopaline synthase terminator was extracted from pBI101 by digesting with SstI and EcoRI.

30 The nucleic acid molecule encoding the PR1-b signal sequence (of SEQ. ID. No. 11) was engineered to have XbaI restriction sites on both ends. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 25 as follows:

- 35 -

atactctaga ccatgggatt ttttctcttt tca

33

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 26
as follows:

5

aggctctagag ttttggccat gagaagagtg

30

The fragment was amplified using pSKG55 as a template (Gopalan et al., "Expression
of the Pseudomonas Syringae Avirulence Protein AvrB in Plant Cells Alleviates its
10 Dependence on the Hypersensitive Response and Pathogenicity (Hrp) Secretion
System in Eliciting Genotype-Specific Hypersensitive Cell Death." Plant Cell
8:1095-1105 (1996), which is hereby incorporated by reference).

PrimeZyme DNA polymerase (Whatman Biometra, Goettingen,
Germany) was used with a hot start procedure for amplification of all fragments. The
15 amplified fragments were purified, digested with the appropriate enzymes, and ligated
into the binary vector pPZP221 (Hajdukiewicz et al., "The Small Versatile pPZP
Family of Agrobacterium Binary Vectors for Plant Transformation," Plant Mol. Bio.
25:989-994 (1994), which is hereby incorporated by reference) or intermediate
constructs, to build up the final constructs. The proper construction of pCPP1294
20 (Figure 1) was confirmed by sequencing on an ABI 377 automated sequencer.

The final constructs were transformed into Agrobacterium tumefaciens
strain GV3101 (Martin et al., "The GUS Reporter System as a Tool to Study Plant
Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a
Reporter of Gene Expression, Academic Press, pp. 23-43 (1992), which is hereby
25 incorporated by reference) by electroporation using a Bio-Rad GenePulser (Bio-Rad
Ltd., York, UK).

**Example 2 - Inoculation with *Peronospora parasitica* Activates *gst1*
Transcription in *Arabidopsis***

30

To evaluate the activity of the *gst1* promoter in a plant other than
potato, transgenic *Arabidopsis* were constructed containing the *E. coli uidA* gene for
 β -glucuronidase (GUS) under control of the *gst1* promoter. Histochemical GUS

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assays of were performed essentially as described by Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp. 23-43 (1992), which is hereby incorporated by reference. Uninoculated and
5 inoculated whole small *Arabidopsis* plants were submerged for 30 minutes on ice in six well microtiter plates in a solution of 1.5% freshly prepared paraformaldehyde in 100 mM sodium phosphate buffer, pH 7.2, containing 0.1% Triton X-100. The plants were washed twice for 5 minutes with sodium phosphate buffer pH 7.2. The plants were then submerged in a solution of 2 mM X-gluc (5-bromo-4-chloro-3-indolyl β-D-
10 glucuronide), 50 mM sodium phosphate, pH 7.2, 0.5% Triton X-100. The solution was vacuum infiltrated into the plants and the plants were then incubated for 16 hours in the dark at 37°C. The staining was stopped by rinsing the plants several times in water and the tissue was then cleared by incubating in several changes of 70% ethanol.
15 Twenty lines were evaluated for GUS expression in uninoculated leaves, leaves inoculated with *Peronospora parasitica* isolate NOCO, and whole plants using a histochemical staining procedure (Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp 23-43 (1992), which is hereby incorporated by reference). Five lines showed more intense staining of the inoculated areas than the uninoculated areas and two lines showed no visible staining of any plant parts except the inoculated leaves (Figure 2). These results are consistent with those reported for potato and reveal that the *gst1* promoter is pathogen inducible in *Arabidopsis*. No induction of GUS activity was detected in
20 the five lines that responded to *P. parasitica* when inoculated with *Pseudomonas syringae* pv. tomato strain DC3000, even after disease symptoms appeared (results not shown). Previously, it was reported that the *gst1* gene is induced in response to fungi, viruses, and nematodes (Strittmatter et al., "Infections with Various Types of Organisms Stimulate Transcription From a Short Promoter Fragment of the Potato
25 *gst1* Gene," Mol. Plant-Microbe Interact. 9:68-73 (1996), which is hereby incorporated by reference), but results with bacterial pathogens were not reported.
30

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Example 3 - Pathogen Inducible Expression of *hrpN* in Transgenic *Arabidopsis*

To generate transgenic *Arabidopsis* expressing *hrpN* in a pathogen-inducible manner, plant transformation vectors, pCPP1292 for cytoplasmic localization of HrpN in plants, and pCPP1294 for extracellular localization of HrpN, were constructed. (Figures 3A and 3B). *Arabidopsis* ecotype Columbia (Col-0) was transformed with the two constructs. *Arabidopsis thaliana* ecotype Columbia (Col-0) plants were grown in a growth chamber at 22° C and a 17 hour photoperiod. Plants with primary fluorescence 5-15 cm tall were transformed via a known vacuum infiltration method (protocol available on the Internet at <http://www.bch.msu.edu/pamgreen/vac.htm>, which is hereby incorporated by reference) adapted from Bechtold et al., *C. R. Acad. Sci. Paris* 316:1194-1199 (1993), and Bent et al., *Science* 265:1856-1860 (1994), which are hereby incorporated by reference. Seeds were collected from each plant individually, sterilized and spread on selection plates containing 150 mg/l gentamycin, 0.2 g/l *Arabidopsis* Growth Medium (Lehle Seeds), and 0.7% Phytagar (Gibco BRL, Bethesda, MD). Plates were vernalized for 2 days at 4°C and then moved to a growth chamber maintained at 22° C and 14 hours light. Gentamycin resistant plants were selected after 2 weeks and individual plants were transplanted to soil. Each individual T1 seedling was brought up by single seed descent and individual plant lines were selected for lack of segregation of gentamycin resistance in the T3 generation. Insertion of T-DNA was confirmed by PCR and Southern analysis.

Transgenic *Arabidopsis* lines were inoculated 2 weeks after sowing with a 5×10^4 conidiospore suspension of *P. parasitica* isolate NOCO. Flats were covered with a humidity dome and moved to the growth chamber maintained at 18° C, 16 hours light, and 100% humidity. Plants were scored for infection 7 days after inoculation with a disease rating system adapted from Cao et al., "Generation of Broad-Spectrum Disease Resistance by Overexpression of an Essential Regulatory Gene in Systemic Acquired Resistance," *Proc. Natl. Acad. Sci. USA* 95:6531-6536 (1998), which is hereby incorporated by reference. A rating of 1, 0 conidiophores present; 2, 0-5 conidiophores present; 3, 6-20 conidiophores on a few leaves; 4, 6-20 conidiophores on all leaves; 5, 20 or more conidiophores present on all leaves. Inoculated leaves were stained with lactophenol-trypan blue (Keogh et al.,

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"Comparison of Histological and Physiological Responses to Phakopsora Pachyrhizi in Resistant and Susceptible Soybean," Trans. Br. Mycol. Soc. 74:329-333 (1980), which is hereby incorporated by reference) to observe the extent of fungal colonization under the microscope.

5 Plants were selected that lacked segregation of antibiotic resistance in the T3 generation. Lines containing the *gst1:hrpN* construct ("GN lines") lines were tested for resistance to *P. parasitica* isolate NOCO in an initial screen.

10 Thirty lines containing the *gst1:signal sequence:hrpN* construct ("GSSN lines") were tested for resistance to *P. parasitica* isolate NOCO in an initial screen. All but one of the lines was free of any signs of the oomycete ten days after inoculation. Ten GSSN lines were chosen for further study and inoculated by spraying with a conidiospore suspension (5×10^4 spores/ml) of *P. parasitica* NOCO. Northern analysis revealed that expression of *hrpN* was induced by *P. parasitica* 2 days after inoculation with strong induction at 4 days (Figure 3A). A range of 15 expression levels were observed among the ten lines, line GSSN 8-4 was chosen for further study as it displayed the highest level of expression. Production of the harpin_{Ea} protein in inoculated plants was confirmed by immuno-blot analysis.

20 RNA was isolated from inoculated plants over a 4 day interval to analyze *hrpN* gene expression. RNA was isolated from 1g of plant tissue as described by Carpenter et al., "Preparation of RNA, in Arabidopsis Protocols," (Martinez-Zapater, JM. and Salinas, J., eds.), Humana Press, Totowa, New Jersey, pp. 85-89 (1998). Twenty micro-gram samples were separated by formaldehyde-agarose gel electrophoresis and blotted onto Hybond N+ membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK). Hybridizations and 25 washing were performed according to Church et al., "Genomic Sequencing," Proc. Natl. Acad. Sci. USA 81:1991-1995 (1984), which is hereby incorporated by reference, using P³² labeled *hrpN* DNA as a probe.

30 The *Arabidopsis* lines GSSN 8-4 (test), Col-0 WT (wild type, control), and Col-0 EV (empty vector, control) were inoculated by drop inoculation with a conidiospore suspension (5×10^4 spores/ml) of *P. parasitica*. Plants were maintained in a growth chamber (16 hours of light, 18° C, 100% humidity) and were scored for infection ten days post inoculation. Nearly all (29 out of 30) 8-4 plants were free of

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any signs of *P. parasitica* (Figure 4A). Trypan blue staining showed that growth of the oomycete was strongly inhibited in GSN 8-4 plants. Extensive hyphal growth was evident in Col-0 WT and Col-0 EV plants (Figure 4B).

Plants were rated for disease severity based on the number of 5 conidiophores per leaf. Nearly all GSN 8-4 plants received a disease rating of 1 with only one being scored 3. The majority of the Col-0 WT and Col-0 EV plants were rated 5, the remainder were rated 4 (Figure 5).

This example demonstrates that pathogen inducible expression of the harpin_{Ea} hypersensitive response elicitor of *Erwinia amylovora* in transgenic plants is 10 a potentially useful strategy for engineering plants for disease resistance. Challenge with *Peronospora parasitica* resulted in accumulation of *hrpN* mRNA, production of harpin_{Ea} protein, and resistance to *P. parasitica*. Upon challenge by *P. parasitica*, it is believed that the transgenic plants most likely mount a hypersensitive response at the site of inoculation, conferring resistance. Subsequently the plants may develop 15 systemic resistance.

For the purposes of the present invention, the *gst1* promoter was most applicable to the *Arabidopsis/P. parasitica* pathosystem since it is well documented that transcription from *gst1* is activated by other oomycete pathogens (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional 20 Activation Selectively upon Fungal Infection," *Mol. Gen. Genet.* 236: (2-3) 179-86 (1993), which is hereby incorporated by reference). Additionally, it has been reported that *gst1* activation is stimulated by ascomycete, viral, and nematode infection and mycorrhization (Strittmatter et al., "Infections with Various Types of Organisms Stimulate Transcription From a Short Promoter Fragment of the Potato *gst1* Gene," 25 *Mol. Plant-Microbe Interact.* 9:68-73 (1996), which is hereby incorporated by reference). Therefore, it is possible that both *gst1:hrpN* and *gst1:signal sequence:hrpN* constructs may also confer resistance against ascomycete, virus, and nematode infection, as well as mycorrhization.

Although the invention has been described in detail for the purpose of 30 illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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All of the references designated as being incorporated herein by reference are intended to be incorporated in their entirety unless specific portions thereof have been identified with particularity.

WHAT IS CLAIMED:

1. A chimeric gene comprising:
a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide,
a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and
a 3' regulatory region operably linked to the first DNA molecule.
2. The chimeric gene according to claim 1 further comprising:
a second DNA molecule encoding a secretion signal polypeptide, the second DNA molecule being operably linked between the promoter and the first DNA molecule.
3. The chimeric gene according to claim 2, wherein the second DNA molecule encodes a secretion signal polypeptide comprising an amino acid sequence of SEQ. ID. No. 11, SEQ. ID. No. 13, SEQ. ID. No. 15, or SEQ. ID. No. 17.
4. The chimeric gene according to claim 3, wherein the second DNA molecule comprises a nucleotide sequence of nt 8-110 from SEQ. ID. No. 10, SEQ. ID. No. 12, SEQ. ID. No. 14, or SEQ. ID. No. 16.
5. The chimeric gene according to claim 1, wherein the promoter is a *gst1* promoter.
6. The chimeric gene according to claim 1, wherein the *gst1* promoter comprises a nucleotide sequence of SEQ. ID. No. 9 or effective fragments thereof.
7. The chimeric gene according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

8. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

9. The chimeric gene according to claim 8, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 3.

10. The chimeric gene according to claim 9, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 4.

11. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

12. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

13. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

14. The chimeric gene according to claim 13, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 1.

15. The chimeric gene according to claim 14, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 2.

16. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

17. The chimeric gene according to claim 16, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 5.

18. The chimeric gene according to claim 17, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 6.

19. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

20. The chimeric gene according to claim 19, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 7.

21. The chimeric gene according to claim 20, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 8.

22. An expression system comprising a vector into which is inserted a chimeric gene according to claim 1.

23. A host cell comprising a chimeric gene according to claim 1.

24. The host cell according to claim 23, wherein the host cell is a bacterial cell or a plant cell.

25. The host cell according to claim 24, wherein the bacterial cell is an *Agrobacterium* cell.

26. The host cell according to claim 24, wherein the host cell is a plant cell.

27. The host cell according to claim 26, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

28. The host cell according to claim 26, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal polypeptide, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

29. The host cell according to claim 26, wherein the promoter is a *gst1* promoter.

30. A transgenic plant resistant to disease resulting from oomycete infection, the transgenic plant comprising:

a chimeric gene according to claim 1, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete.

31. The transgenic plant according to claim 30, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

32. The transgenic plant according to claim 31, wherein the second DNA molecule encodes a secretion signal polypeptide comprising an amino acid sequence of SEQ. ID. No. 11, SEQ. ID. No. 13, SEQ. ID. No. 15, or SEQ. ID. No. 17.

33. The transgenic plant according to claim 32, wherein the second DNA molecule comprises a nucleotide sequence of nt 8-110 from SEQ. ID. No. 10, SEQ. ID. No. 12, SEQ. ID. No. 14, or SEQ. ID. No. 16.

34. The transgenic plant according to claim 30, wherein the *gst1* promoter comprises a nucleotide sequence of SEQ. ID. No. 9 or effective fragments thereof.

35. The transgenic plant according to claim 30, wherein the oomycete is a species of *Plasmopara*, *Phytophthora*, *Peronospora*, *Pseudoperonospora*, *Bremia*, *Sclerospora*, *Aphanomyces*, *Pythium*, or *Albugo*.

36. The transgenic plant according to claim 30, wherein the transgenic plant is selected from a group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

37. The transgenic plant according to claim 36, wherein the transgenic plant is a grape plant.

38. The transgenic plant according to claim 37, wherein the oomycete is selected from the group consisting of *Plasmopara viticola* and *Phytophthora parasitica*.

39. The transgenic plant according to claim 36, wherein the transgenic plant is a tobacco plant.

40. The transgenic plant according to claim 39, wherein the oomycete is selected from the group consisting of *Peronospora tabacina*, *Pythium* spp., and *Phytophthora* spp.

41. The transgenic plant according to claim 30, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

42. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

43. The transgenic plant according to claim 42, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 3.

44. The transgenic plant according to claim 44, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 4.

45. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

46. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

47. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

48. The transgenic plant according to claim 47, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 1.

49. The transgenic plant according to claim 48, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 2.

50. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

51. The transgenic plant according to claim 50, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 5.

52. The transgenic plant according to claim 51, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 6.

53. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

54. The transgenic plant according to claim 53, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 7.

55. The transgenic plant according to claim 54, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 8.

56. The transgenic plant according to claim 30, wherein the chimeric gene is stably inserted into the genome of the transgenic plant.

57. A method of making a recombinant plant cell comprising: transforming a plant cell with a chimeric gene according to claim 1 under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter.

58. A method of making a plant resistant to disease resulting from oomycete infection, the method comprising:

transforming a plant cell with a chimeric gene according to claim 1 under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and
regenerating a plant from the transformed plant cell.

59. The method according to claim 58, wherein said transforming is performed under conditions effective to insert the chimeric gene into the genome of the plant cell.

60. The method according to claim 58, wherein said transforming is *Agrobacterium* mediated.

61. The method according to claim 58, wherein said transforming comprises:

propelling particles at the plant cell under conditions effective for the particles to penetrate into the cell interior and

introducing an expression vector comprising the chimeric gene into the plant cell interior.

62. The method according to claim 58, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

63. The method according to claim 58, wherein the promoter is a *gst1* promoter.

64. The method according to claim 58, wherein the oomycete is a species of *Plasmopara*, *Phytophthora*, *Peronospora*, *Pseudoperonospora*, *Bremia*, *Sclerospora*, *Aphanomyces*, *Pythium*, or *Albugo*.

65. The method according to claim 58, wherein the transgenic plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

66. The method according to claim 65, wherein the transgenic plant is a grape plant.

67. The method according to claim 66, wherein the oomycete is selected from the group consisting of *Plasmopara viticola* and *Phytophthora parasitica*.

68. The method according to claim 65, wherein the transgenic plant is a tobacco plant.

69. The method according to claim 68, wherein the oomycete is selected from the group consisting of *Peronospora tabacina*, *Phytophthora* spp., and *Pythium* spp.

70. The method according to claim 58, wherein the hypersensitive response elicitor protein or polypeptide derives from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

71. A transgenic plant seed obtained from the transgenic plant according to claim 30.

72. A transgenic plant scion or rootstock cultivar obtained from the transgenic plant according to claim 30.



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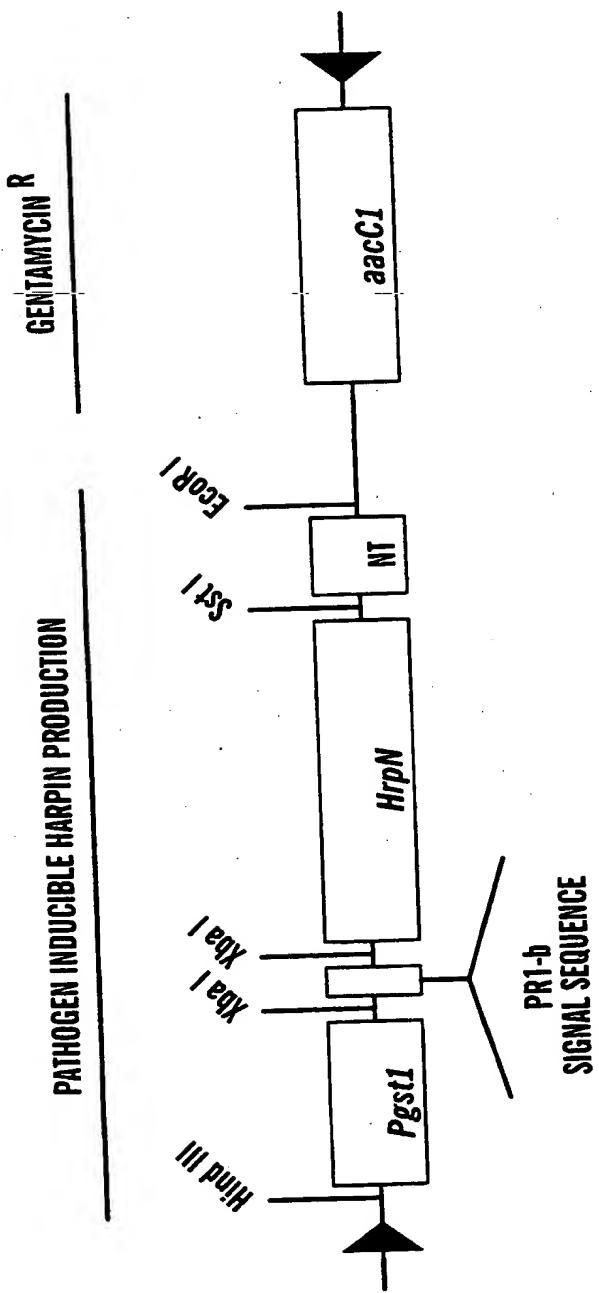


FIG. 1



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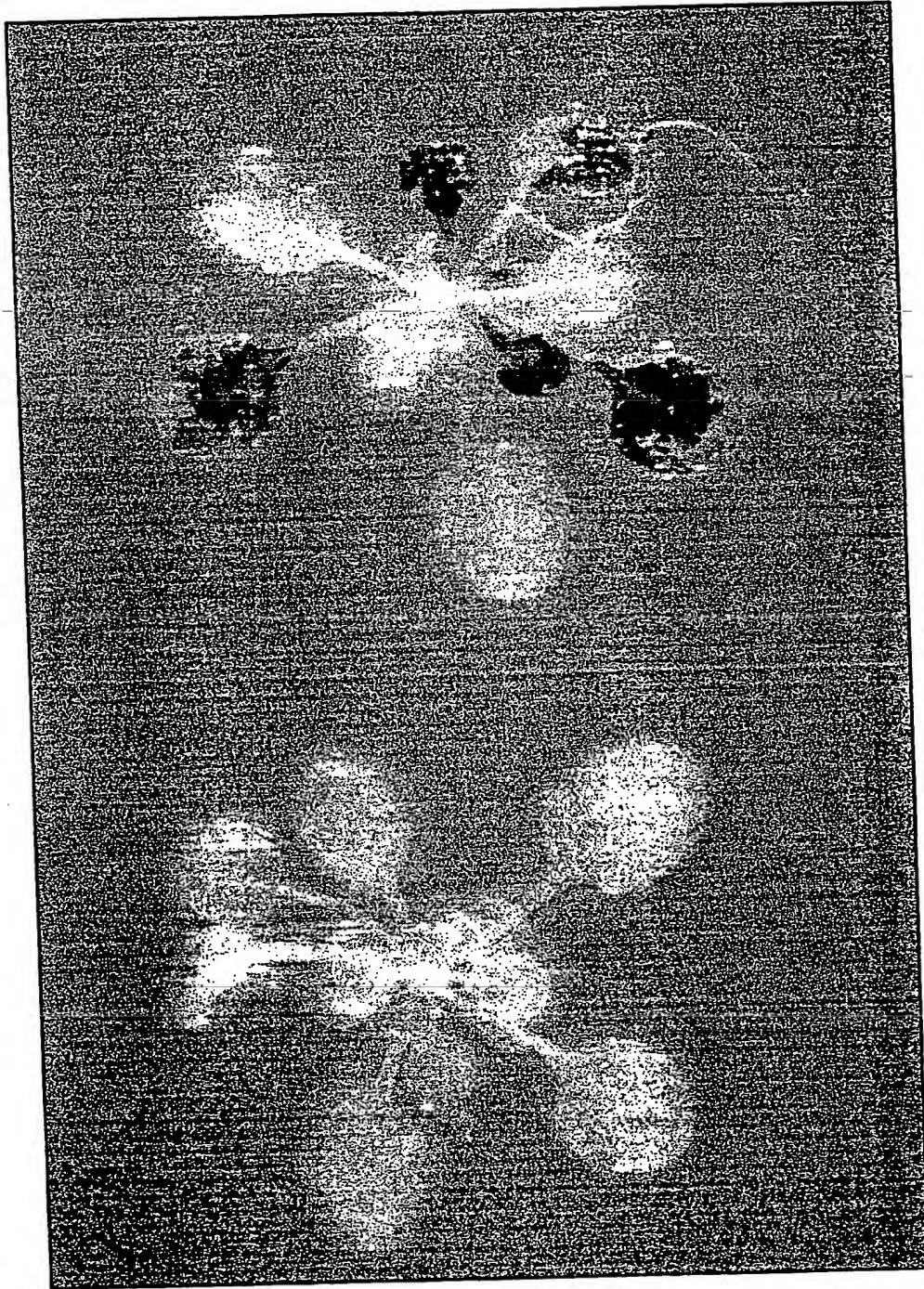


FIG. 2



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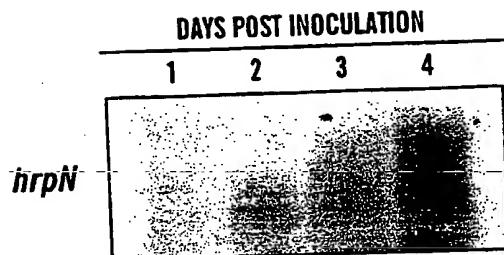


FIG. 3A

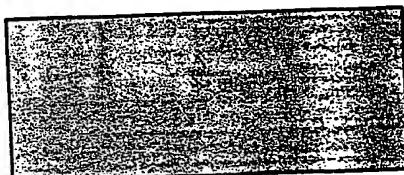


FIG. 3B

4/6

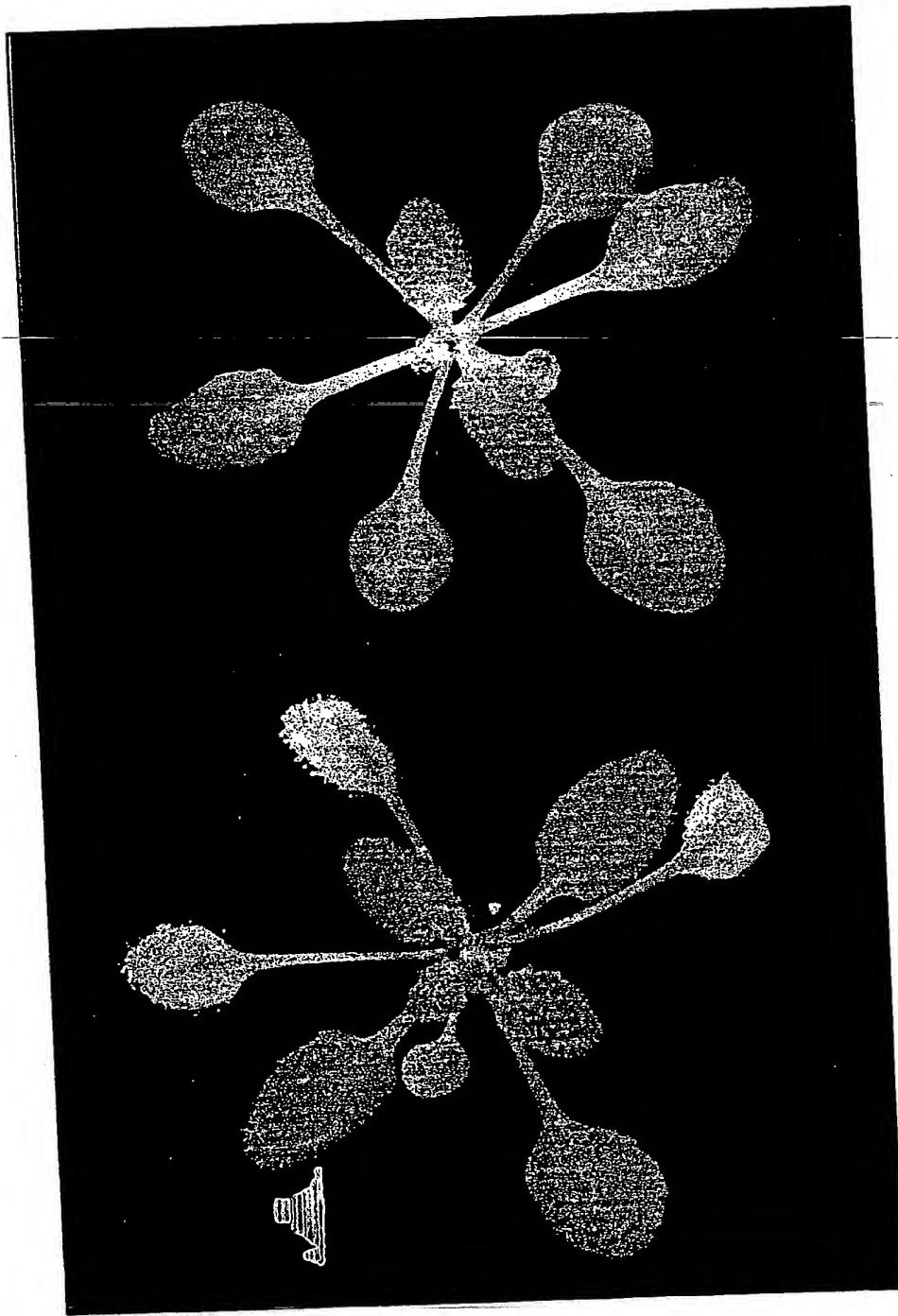


FIG. 4A



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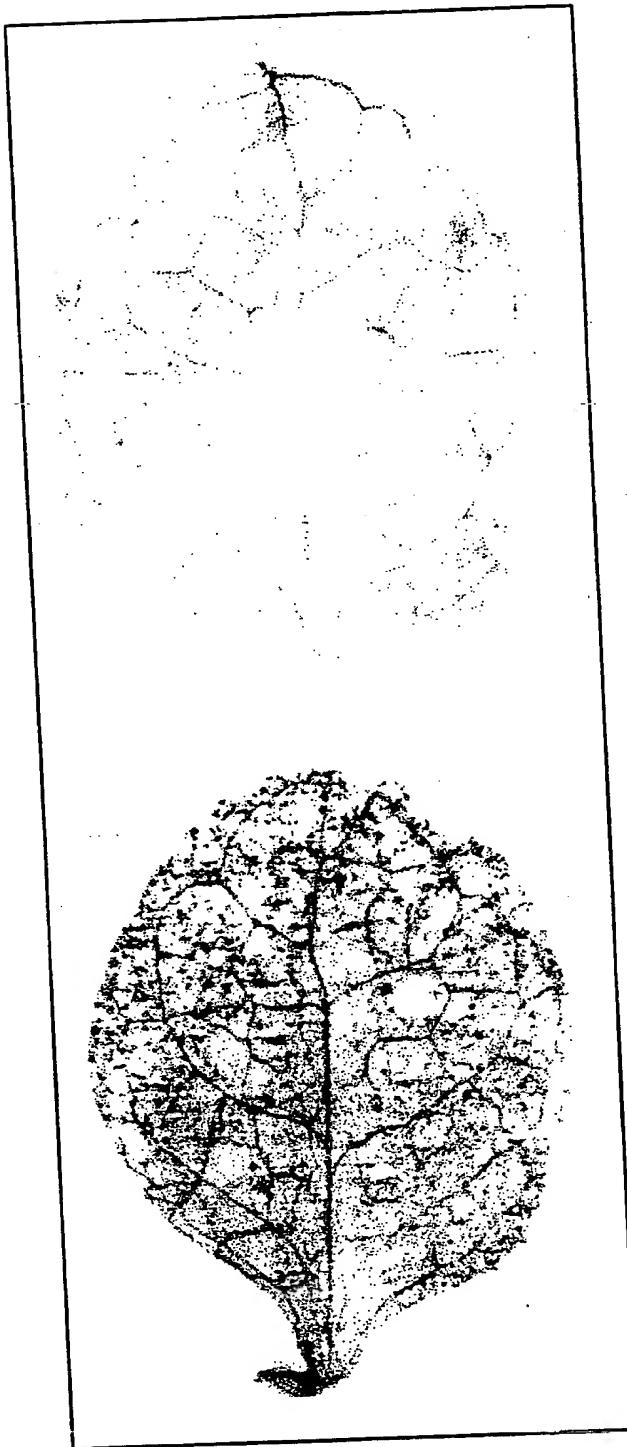


FIG. 4B



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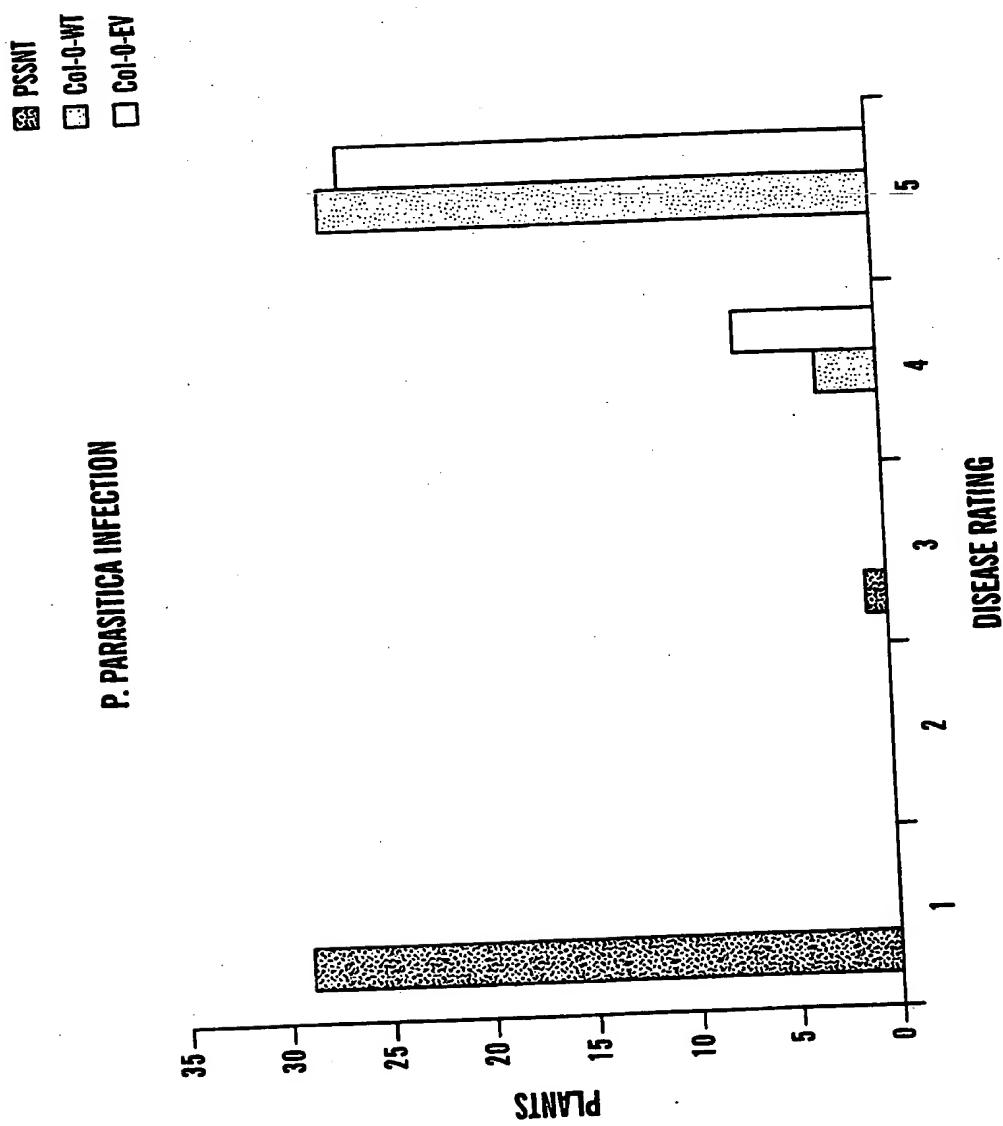


FIG. 5



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SEQUENCE LISTING

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<120> OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF
PATHOGEN-INDUCED EXPRESSION OF A HETEROLOGOUS
HYPERSENSITIVE RESPONSE ELICITOR

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			60
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			75
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<211> 341

<212> PRT

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5

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20

25

30

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35

40

45

Arg	Asn	Gly	Gln	Leu	Asp	Asp	Ser	Ser	Pro	Leu	Gly	Lys	Leu	Leu	Ala

50

55

60

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65

70

75

80

Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe

85

90

95

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100

105

110

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115

120

125

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130

135

140

Leu	Asn	Lys	Ile	Ala	Gln	Phe	Met	Asp	Asp	Asn	Pro	Ala	Gln	Phe	Pro

145

150

155

160



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<211> 344

<212> PRT

<213> Pseudomonas solanacearum

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 35 40 45

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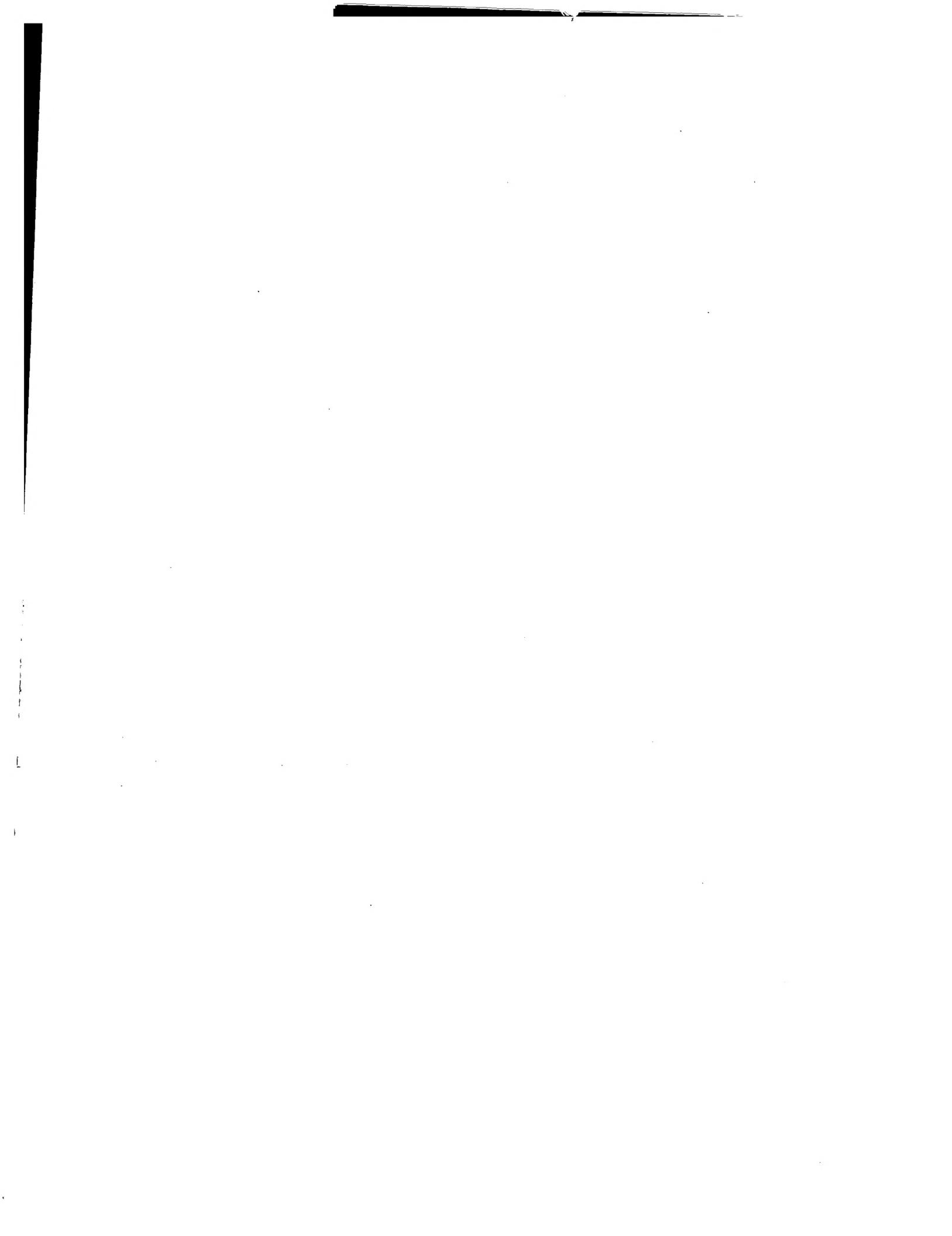
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 180 185 190
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 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
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<210> 11
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<210> 12
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<210> 13
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Ser Gln

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<400> 14



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 <211> 25
 <212> PRT
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<210> 19
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<223> Description of Artificial Sequence: primer

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<210> 22
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<210> 23
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 <223> Description of Artificial Sequence: primer

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 <223> Description of Artificial Sequence: primer

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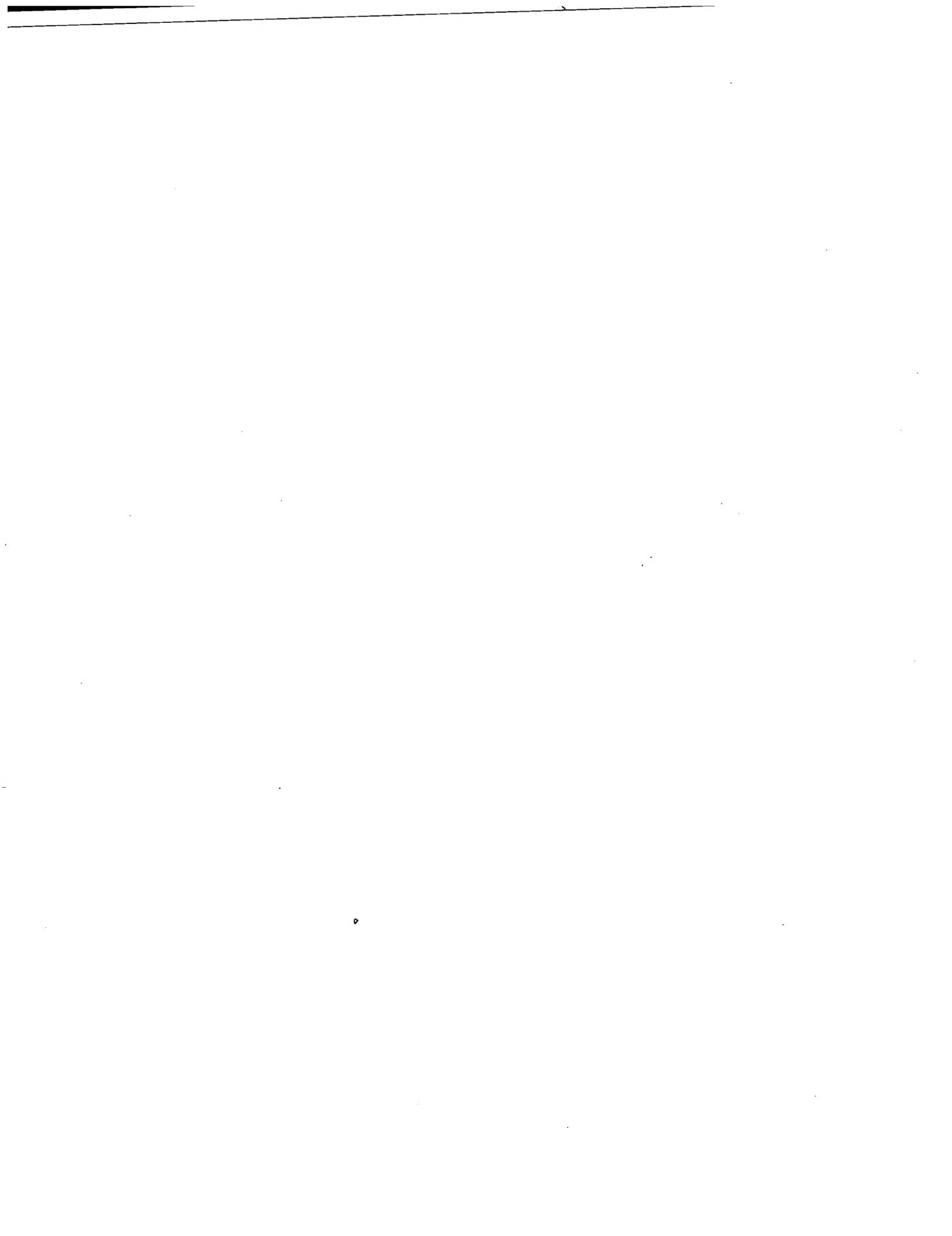
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<210> 26
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 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: primer



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<400> 26
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INTERNATIONAL SEARCH REPORT

PCT/US01/02579

Classification of subject matter
 IPC: C12N 5/04; 15/09, 15/29, 15/31, 15/82; A01H 5/00
 US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

R. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 435/69.1, 320.1, 419, 468; 536/23.1, 23.6, 23.7, 24.1; 800/278, 279, 287, 288, 298, 295

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 STN CAS, WEST2.0
 search terms: hypersensitive, elicitor, oomycete, gtl promoter, transgenic plants , disease resistance, fungal pathogen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,859,332 A (STRITTMATTER et al) 12 January 1999 (12.01.99), see entire document, especially columns 3-5, 11-18, 23-26.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72
A	HART et al. Regulated Inactivation of Homologous Gene Expression in Transgenic Nicotiana sylvestris Plants Containing a Defense-related Tobacco Chitinase Gen. Mol. Gene. Genet. 1992, Vol. 235, No 2-3, pages 179-186, see entire document.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72
Y	WEI et al. Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia amylovora. Science 03 July 1992, Vol. 257, pages 85-88, see entire document.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72

Further documents are listed in the continuation of Box C.

See patent family annex.

- Special categories of cited documents:
- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
 - *A* document defining the general state of the art which is not considered to be of particular relevance
 - *E* earlier document published on or after the international filing date
 - *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 - *O* document referring to an oral disclosure, use, exhibition or other means
 - *P* document published prior to the international filing date but later than the priority date claimed

- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

14 MAY 2001

Date of mailing of the international search report
14 JUN 2001

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 Commissioner of Patents and Trademarks
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 Washington, D.C. 20231
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Authorized officer
MEDINA A. IBRAHIM
 Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/02579

A. CLASSIFICATION OF SUBJECT MATTER:

US CL.:

435/69.1, 320.1, 419, 468; 536/23.1, 23.6, 23.7, 24.1; 800/278, 279, 287, 288, 298, 295

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a specified *gst1* promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.

Group II, claim(s) 1-4, 22-26, 28, 31-33, 62, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a second DNA molecule encoding specific sequences of secretion signal polypeptides, a *gst1* promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.

Group III, claim(s) 1, 7-21, 22-26, 27, 41-55, 70, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide of a specified sequence, a *gst1* promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: